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PROGRAMA DE PÓS-GRADUAÇÃO em
Ciências Ambientais

LESLIE LISSETHE MORALES ESPINOZA

Ecotoxicological aspects of microplastic PET (Polyethylene Thereftalate) in freshwater using biochemical biomarkers from *Danio rerio*

Sorocaba
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Orientadora: Prof. Dra. Renata Fracácio Francisco
Coorientadora: Prof. Dra. Cleoni dos Santos Carvalho

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"What we know is a drop, what we don't know is an ocean."

— Isaac Newton

Resumo

Os microplásticos (MPs) representam contaminantes emergentes que têm atraído extensa atenção da investigação devido à sua prevalência tanto em ambientes naturais como urbanos. Entre estes ambientes, os ecossistemas de água doce foram significativamente impactados. No entanto, atualmente faltam níveis de concentração seguros estabelecidos para MPs. Assim, através de uma revisão bibliográfica da abundância e distribuição de MPs nestes ecossistemas, bem como da sua relação com os estudos ecotoxicológicos mais recentes, foi possível fazer uma análise de risco e propor níveis seguros de MPs para ambientes de água doce (PNEC: 0,0027 mg L^{-1} ou 2,61 partículas L^{-1}). Isto foi estimado para 7 tipos de MPs (PE, PS, PVC, PA6 e PES, PET e copolímero EEA) que foram baseados na revisão ecotoxicológica da pesquisa. Posteriormente, reconhecendo lacunas na literatura relativas a tipos específicos de MP, o presente estudo concentrou-se na investigação da ecotoxicidade de MPs de tereftalato de polietileno (PET-MPs). Com base nessas informações, foram realizados dois experimentos crônicos de 21 dias com *Danio rerio* para estudar sua ecotoxicidade isolada e misturada com cádmio (Cd). O primeiro teste consiste na comparação dos diferentes tamanhos do PET-MP isolado (300 - 450 µm; 53 - 75 µm); e o segundo da sua mistura (53 - 75 µm) com Cd. Concentrações ambientalmente relevantes de MPs (983 partículas. L^{-1} e 330 partículas. L^{-1}) e Cd (10 ug L^{-1}) foram utilizadas durante os testes de laboratório. Análises bioquímicas foram realizadas para observar a toxicidade em termos de estresse oxidativo (proteínas carboniladas), metabolismo (glicose e triglicerídeos) e atividade enzimática (malato desidrogenase e lactato desidrogenase). Especificamente, no caso do experimento de diferentes tamanhos e concentrações de PET-MP, os níveis de glicose, CP, MDH e LDH exibiram que partículas maiores têm efeitos mais pronunciados em comparação com as menores, e concentrações mais altas produziram similarmente maior impacto. No segundo experimento envolvendo a combinação de PET-MPs e Cd, foi observada toxicidade aumentada em termos de níveis de estresse oxidativo e atividade enzimática como resultado das análises de CP, LDH e MDH.

Palavras-chave: Microplásticos, Água doce, Ecotoxicidade, Análise de risco, Análise bioquímica.

Abstract

Microplastics (MPs) represent emerging contaminants that have garnered extensive research attention due to their prevalence in both natural and urban settings. Among these environments, freshwater ecosystems have been significantly impacted. However, established safe concentration levels for MPs are currently lacking. Thus, by a bibliographical review of the abundance and distribution of MPs in these ecosystems, as well as their relationship with the most recent ecotoxicological studies, it was possible to make a risk analysis and to propose safe MPs levels for freshwater environments (PNEC: 0.0027 mg L^{-1} or 2.61 particles L^{-1}). This was estimated for 7 types of MPs (PE, PS, PVC, PA6, and PES, PET, and EEA copolymer) that were founded on the ecotoxicology review of the survey. Subsequently, recognizing gaps in the literature pertaining to specific MP types, the present study focused on investigating the ecotoxicity of Polyethylene terephthalate MPs (PET-MPs). Based on this information, two 21-day chronic experiments were carried out with *Danio rerio* to study its ecotoxicity isolated and mixed with cadmium (Cd). The first test consists of the comparison of the different sizes of isolated PET-MP (300 - 450 µm; 53 - 75 µm); and the second of its mixture (53 - 75 µm) with Cd. Environmentally relevant concentrations of MP (983 particles. L^{-1} and 330 particles. L^{-1}) and Cd (10 ug L^{-1}) were used during laboratory tests. Biochemical analyzes were performed to observe toxicity in terms of oxidative stress (carbonylated proteins), metabolism (glucose and triglycerides), and enzymatic activity (malate dehydrogenase and lactate dehydrogenase). Specifically, in the case of the experiment of different sizes and concentrations of PET- MP, Glucose, CP, MDH, and LDH levels exhibited that larger particles have more pronounced effects compared to smaller ones, and higher concentrations similarly yielded greater impact. In the second experiment involving the combination of PET-MPs and Cd, heightened toxicity was observed in terms of oxidative stress levels and enzymatic activity as a result of CP, LDH, and MDH analyses.

Keywords: Microplastics, Freshwater, Ecotoxicity, Risk analysis, Biochemical analysis.

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List of Acronyms

ABNT:	Associação Brasileira de Normas Técnicas
AF:	Assessment Factor
BSA:	Bovine serum albumin
CAPES:	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEUA:	Comissão de Ética no Uso de Animais
Cd:	Cadmium
CONAMA:	Environmental National Council of Brazil
CP:	Carbonylated protein
DNPH:	Dinitrophenylhydrazine
EC_{50}	Effect Concentration of 50% of the organism
ICTS:	Instituto de Ciência e Tecnologia - Câmpus de Sorocaba
IFPS:	Federal Institute of São Paulo
HONEC:	Highest Observed No Effect Concentration
LC_{50}	Lethal Concentration of 50% the organism
LDH:	Lactate dehydrogenase
LOEC:	Lowest Observed Effect Concentration
MEC:	measured environmental concentrations)
MDH:	Malate dehydrogenase
MPs:	Microplastics
NPs:	Nanoplastics
PE:	Polyethylene
PES:	Polyester
PET:	Polyethylene Terephthalate

PET-MPs:	Polyethylene Terephthalate microplastics
PNEC:	Predicted no-effect concentration
PP:	Polypropylene
PS:	Polystyrene
PUR:	Polyurethane
PVC:	Polyvinylchloride
PA:	Polyamide
OECD:	Organization for Economic Co-operation and Development
RQ:	Risk Quotient
SEM:	Scanning electron microscopies
SSD:	Species Sensitivity Distribution
UNESP:	Universidade Estadual Paulista "Júlio de Mesquita Filho"
UFSCar:	Universidade Federal de São Carlos

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Introduction

The modern era has witnessed the prolific production and introduction of diverse plastic polymers into the environment. Notable examples include polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polystyrene (PS), and polyurethane (PUR). The production of these polymers escalated significantly, increasing by twentyfold between 1964 and 2015, amounting to an estimated 322 million metric tons. Alarmingly, projections suggest that this figure could double by 2035 and quadruple by the 2050s (STAP, 2018).

Ritchie & Roser (2018) estimated that there were more than 5 trillion plastic particles in the world's surface waters in 2018. Bringing concern abroad about the degradation of these compounds that are made through small pieces of plastic, characterized as microplastics (MPs) or nanoplastics (NPs) (depending on their size) (Nkwachukwu et al., 2013). These residues remain in environments available to interact with every life form, triggering ecotoxicological repercussions in different ecosystems (Bajt et al., 2021).

The effects of MPs extend to a wide variety of aquatic organisms, not only due to the physical damage caused by their ingestion but also due to their toxicity or bioaccumulation of certain associated chemicals (Horton & Dixon, 2018), including negative effects on the rates of growth, survival and fertility, feeding inhibition, genotoxicity, inflammation, and tumor promotion (Pico et al., 2019; GESAMP, 2015). All these effects represent a great risk for this type of organism and unfortunately is quite difficult to assess and/or quantify, due to the diversity of existing types, properties, sizes, formats, ecotoxicological biomarkers, and impact assessment methods for MPs (Koelmans et al., 2022). Another relevant fact to consider is that most of the risk assessments have been carried out for marine environments, leaving aside freshwater environments, despite the fact that the quantity of MPs in this medium is almost the same as the one on the marine environment (Peng et al., 2017; Li et al., 2018). That is why it is extremely important to give greater emphasis to this type of risk analysis, for the sake of understanding what really happens in these ecosystems and thus be able to establish a safe level of MPs for the protection of the species that inhabit it.

Ecotoxicological studies carried out with MPs have mainly focused on PS, hence there is a great gap in the ecotoxicological effects produced by other types of MPs such as PET (Bashirova et al. 2023). PET is a widely used type of plastic worlwide, and in Europe it represents a percentage of 7.1% of all plastics used (Plastics Europe, 2022). Currently, it already constitutes potential risks to human health, since some studies such as that of Harvey and Watts (2018), demonstrated the existence of PET-MPs in human feces. Due to these alarming data, it is also essential to study what happens to them in aquatic environments and what possible effects they may cause on the organisms that inhabit them.

Another important factor is the MPs joint exposure with other xenobiotics, since it is presumed that it could increase their bioavailability, as well as their toxicity (Zhang et al. 2020). This is a matter of great concern since the coexistence of MPs and other compounds has already been occurring in the environment for many years (Yang et al. 2022). Some factors, such as the type of surface, and the size vary its ability to adsorb other contaminants, such as metallic and organic compounds and some pharmaceuticals (Zhang et al., 2022). In the case of heavy metals, they represent a greater risk because they easily can be adsorbed from MPs (Zhou et al., 2019). A realistic scenario of this phenomenon was investigated by Deng et al., 2020, who studied the interaction of heavy metals with MPs in mangrove sediments, determining that the most abundant MP in the study area was PET and due to the characteristics of its surface it was able to adsorb heavy metals such as Cr, Zn, Pb, and Cd.

Biomarkers are defined as quantitative measures of changes in the biological system related to the toxic effects of some chemicals that exist in nature (Torres et al. 2008). Within the wide variety of them, biochemical biomarkers allow us to understand aspects of the chemical environment that cannot be made just by quantifying pollutants, such as bioavailability, interactions between other chemicals, long-term effects, and others. (Venturino & Pechen, 2005). To date, and according to various biochemical analyzes carried out in ecotoxicological trials, it has been shown that MPs, depending on their size and dose, can induce oxidative damage (increased lipid peroxidation and DNA strand breaks), alter the antioxidant system and metabolism (Prokić et al., 2019).

So, in view of what was previously described, this research had two main objectives: 1) Contribute with data on abundance, distribution and ecotoxicological aspects of MPs, as well as establish possible safe environmental concentration for microplastics in freshwater; 2) To evaluate the potential effects of PET secondary MPs at different particle sizes, doses and combination with cadmium, measured with biochemical biomarkers of metabolism, enzymatic activity, and oxidative stress of *Danio rerio*.

In order to facilitate the understanding of this work, they were divided into the following chapters:

Chapter 1 – Review article: ***Microplastics in Freshwater Ecosystems: Occurrence, Ecotoxicological Aspects, and Ecological Risk Analysis.*** Planned to be submitted in the periodic "Ecotoxicology". The first chapter aims to collect data from the literature on MPs found in freshwater ecosystems around the world, discussing the types, size, abundance, and distribution, in addition to the bibliographic survey on the tests carried out with different types of MPs and different groups of organisms and a final Risk analysis that includes the predicted no effect concentration (PNEC) and the risk quotient (RQ).

Chapter 2 – Research article: ***Ecotoxicological studies of secondary microplastic (PET) in freshwaters using biochemical biomarkers from Danio rerio: emphasis on granulometric conditions, environmental concentrations and co-exposure with cadmium***. Intend to be published in the periodic "Environmental Pollution". This work has two main objectives, evaluated through biochemical biomarkers of metabolism (total proteins, glucose and triglycerides), oxidative stress (protein carbonylation) and enzymatic activity (lactate dehydrogenase and malate dehydrogenase) throughout two different experiments: (1) To determinate if there is ecotoxicological codependency between different sizes (53-75 µm, 350-400 µm) and environmentally relevant concentrations (330 particles. L^{-1} and 938 particles. L^{-1}) of the isolated PET-MP; (2) To establish if the effects of PET-MPs are potentiated with the addition of Cd at theoretically concentration safe levels for the protection of aquatic life, based on CONAMA 357 legislacion (10 µg. L^{-1}).

REFERENCES

Bajt, O. (2021). From plastics to microplastics and organisms. *FEBS Open Bio*, *11*(4), 954–966. https://doi.org/10.1002/2211-5463.13120

Deng, J., Guo, P., Zhang, X., Su, H., Zhang, Y., Wu, Y., & Li, Y. (2020). Microplastics and accumulated heavy metals in restored mangrove wetland surface sediments at Jinjiang Estuary (Fujian, China). *Marine Pollution Bulletin*, *159*(March), 111482. https://doi.org/10.1016/j.marpolbul.2020.111482

GESAMP. (2015). *Sources, fate and effects of microplastics in the marine environment: a global assessment*.

Horton, A. A., & Dixon, S. J. (2018). Microplastics: An introduction to environmental transport processes. *Wiley Interdisciplinary Reviews: Water*, *5*(2), e1268. https://doi.org/10.1002/wat2.1268

Koelmans, A. A., Redondo-Hasselerharm, P. E., Nor, N. H. M., de Ruijter, V. N., Mintenig, S. M., & Kooi, M. (2022). Risk assessment of microplastic particles. *Nature Reviews Materials*, *7*(2), 138–152. https://doi.org/10.1038/s41578-021-00411-y

Nkwachukwu, O., Chima, C., Ikenna, A., & Albert, L. (2013). Focus on potential environmental issues on plastic world towards a sustainable plastic recycling in developing countries. *International Journal of Industrial Chemistry*, *4*(1), 34. https://doi.org/10.1186/2228-5547-4-34

Peng, J., Wang, J., & Cai, L. (2017). Current understanding of microplastics in the environment: Occurrence, fate, risks, and what we should do. *Integrated Environmental Assessment and Management*, *13*(3), 476–482. https://doi.org/10.1002/ieam.1912

Pico, Y., Alfarhan, A., & Barcelo, D. (2019). Nano- and microplastic analysis: Focus on their occurrence in freshwater ecosystems and remediation technologies. *TrAC - Trends in Analytical Chemistry*, *113*, 409–425. https://doi.org/10.1016/j.trac.2018.08.022

Prokić, M. D., Radovanović, T. B., Gavrić, J. P., & Faggio, C. (2019). Ecotoxicological effects of microplastics: Examination of biomarkers, current state and future perspectives. *TrAC - Trends in Analytical Chemistry*, *111*, 37–46. https://doi.org/10.1016/j.trac.2018.12.001

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*(3), 849–873. https://doi.org/10.1111/brv.12480

Ritchie, H., & Roser, M. (2018). *Our World in Data*. Plastic Pollution. https://ourworldindata.org/plastic-pollution#how-does-plastic-impact-wildlife-and-human-health

STAP. (2018). *Plastics and the circular economy*.

Torres, M. A., Barros, M. P., Campos, S. C. G., Pinto, E., Rajamani, S., Sayre, R. T., & Colepicolo, P. (2008). Biochemical biomarkers in algae and marine pollution: A review. *Ecotoxicology and Environmental Safety*, *71*(1), 1–15. https://doi.org/10.1016/j.ecoenv.2008.05.009

Venturino, A., & Pechen de D’Angelo, A. M. (2005). Biochemical targets of xenobiotics: Biomarkers in amphibian ecotoxicology. *Applied Herpetology*, *2*(3), 335–353. https://doi.org/10.1163/1570754054507433

Yang, H., Zhu, Z., Xie, Y., Zheng, C., Zhou, Z., Zhu, T., & Zhang, Y. (2022). Comparison of the combined toxicity of polystyrene microplastics and different concentrations of cadmium in zebrafish. *Aquatic Toxicology*, *250*(July), 106259. https://doi.org/10.1016/j.aquatox.2022.106259

Zhang, R., Wang, M., Chen, X., Yang, C., & Wu, L. (2020). Combined toxicity of microplastics and cadmium on the zebrafish embryos (*Danio rerio)*. *Science of the Total Environment*, *743*, 140638. https://doi.org/10.1016/j.scitotenv.2020.140638

Zhang, T., Jiang, B., Xing, Y., Ya, H., Lv, M., & Wang, X. (2022). Current status of microplastics pollution in the aquatic environment, interaction with other pollutants, and effects on aquatic organisms. *Environmental Science and Pollution Research*, *29*(12), 16830–16859. https://doi.org/10.1007/s11356-022-18504-8

Zhou, Y., Liu, X., & Wang, J. (2019). Characterization of microplastics and the association of heavy metals with microplastics in suburban soil of central China. *Science of the Total Environment*, *694*. https://doi.org/10.1016/j.scitotenv.2019.133798

Chapter 3

Final Considerations and Future Perspectives

The foundation of this research was built upon a series of intricate questions that underscored the complexity of the challenges posed by MPs. These inquiries were not easily answered, questions such as, "Can we accurately quantify the risk posed by microplastics in our surroundings?" and "Is there a well-defined safe threshold to safeguard aquatic life?" exemplify the uncertainty that looms over this subject. Additionally, the exploration extended to the behavior of different types of MPs—do they all elicit the same effects across various species? The concurrent presence of other pollutants alongside MPs, along with the potential for intensification of ecotoxicological impacts due to MPs' size, format, and origin, further deepened the complexity of the matter. It was within these gaps, borne from the limitations of existing literature, that the two chapters of this study found their genesis. The research endeavors to shed light on these intricate questions and contribute to a more comprehensive understanding of the multifaceted challenges presented by microplastics in freshwater ecosystems.

The first chapter grapples with the absence of established safe levels for MPs and embarks on a daunting task: conducting a risk analysis for these particles. This undertaking is not without its challenges, primarily stemming from the unique nature of microplastics as pollutants. Unlike conventional molecules, microplastics exhibit a remarkable degree of complexity owing to their diverse array of characteristics—ranging from types, densities, physicochemical properties, and surface area, to sizes and origins.This research decided to first collect information about what is found naturally in the freshwater environments, and from this, relate it to ecotoxicology, and verify if what is being tested is what really causes problems in nature. Different parameters were considered, such as size, format, origin, and type of MPs. Throughout our search, it was indeed verified that most of the ecotoxicological studies do not test what is more common to be found in nature. Although each type, format, and size of MPs is important, greater efforts should be put into studying those gaps that would allow the establishment of regulations in the future. Secondly, the present work proposed a safe level, based on all the sizes, types, and formats that could be found in ecotoxicological studies. All of this was elaborated based in the fact that freshwater, MPs of different characteristics will be in constant movement due to the dynamics of these ecosystems, representing a risk for organism from all the different depths (benthic and pelagic).

In the second chapter, another notable blank space emerged, partly due to the comprehensive exploration undertaken in the first chapter: the paucity of PET studies with freshwater species such as *D. rerio*. This may be because the production of MPs for ecotoxicological studies is not very common, since it is usual to work with MPs of the primary type, which are easily acquired from factories. However, they did not experience the processes, that normally MPs go through in nature. Also, in addition to choosing the PET for the development of an ecotoxicological study, it was compared different sizes, and concentrations, to see if the effects varied or not, since in some studies certain correlations between sizes, formats, and concentrations were observed.

Another particularity that was observed, in the case of some studies, was the association with other pollutants. Greater effects were seen than those obtained when some type of MPs was tested alone. For this reason, it was decided to test a metal in a dose that is theoretically safe for aquatic life (10 µg. L^{-1}), as this is established in Brazilian regulations (CONAMA 357). Thus, the outcomes of this research effectively validated the trends observed through the analysis of biochemical biomarkers associated with oxidative stress and enzymatic activity, even with safe concentration levels. Results confirmed that both the size and the concentrations are factors to consider when talking about the ecotoxicity of PET-MPs and is one more piece of evidence, as well as those studies carried out with other types of MPs, in which this codependency is demonstrated.

As already mentioned, MPs are complex substances, therefore, based on what has been obtained, it is imperative that future research in the field of ecotoxicology focus on amassing a more extensive dataset regarding the ecotoxicological impacts of various types of MPs, including those that have not yet undergone thorough investigation .The different shapes should not be neglected either, fibers are another abundant MP shape in nature, important to be further analyzed in the future. In addition, more environmental concentrations should be tested, since each aquatic environment presents its own levels of MPs. Unfortunately, as microplastic contamination escalates due to the continuous and increasing utilization of plastic materials, understanding the effects of MPs at varying concentrations becomes even more crucial, that is why the scientific community can shed light on the intricate relationships between microplastics and aquatic ecosystems, paving the way for more informed environmental management strategies in the face of mounting plastic pollution.